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The comparison of biophysical properties of DB-67 and its ester DB-67-4ABTFA determined by fluorescence spectroscopy methods

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ABSTRACT

Fluorescence spectroscopy methods are applied to the study of camptothecin analogue DB-67 and its ester DB-67-4ABTFA (trifluoroacetic acid salt of 20(*S*)-aminobutyrate substituted DB-67). Camptothecin and many of its analogues exhibit anticancer properties. They are fluorescent compounds, so using the method of fluorescence anisotropy measurements and fluorescence spectra recording many biophysical properties can be determined including affinity to proteins and membranes. One can also observe the process of conversion of the ester into DB-67. Active lactone form of camptothecin in fluids at pH 7.4 hydrolyses and converts into inactive carboxylate. Process of camptothecin deactivation is accelerated in plasma and after about 2 h the total conversion to carboxylate form occurs. It is caused by fast and irreversible binding of carboxylate form to the human serum albumin (HSA). Camptothecin carboxylate bound to HSA does not lactonise. On the other hand, camptothecin lactone binding to membranes is reversible, but as long as lactone form bound to membranes does not hydrolyse. Knowledge of binding properties to proteins and membranes permits to select among many camptothecin analogues the ones exhibiting desirable behavior in physiological conditions: high affinity of lactone form to membranes and low affinity of carboxylate form to albumin. The studied DB-67 and DB-67-4ABTFA fulfill these requirements.

1. Introduction

Camptothecin (CPT, alkaloid obtained from the tree *Camptotheca acuminata*) and its anticancer properties, which follow from inhibition of topoisomerase I–DNA complex in replication process, are known from the 50s of the last century (Wall et al., 1966). A serious limitation of application of CPT and its analogues in anticancer chemotherapy is hydrolysis of these compounds which occur in physiological conditions. In solutions of pH > 5.5 biological active lactone form of CPT undergoes transformation into inactive carboxylate form, see Kohn and Pommier (2000). The chemical structures of the both forms of camptothecin are presented in Fig. 1A. Knowing the kinetics of camptothecin deactivation allow to evaluate the stability of a new analogues of CPT in physiological fluids. It seems that the affinities of this compounds to albumin and cell membranes have the decisive impact on the stability and activity of CPTs. Lactone form molecules of camptothecins bound to membranes do not hydrolyse (Burke et al., 1993), so high affinity of CPTs to cell membranes is a desirable property. On the other hand, because of preferential and non-reversible binding of carboxylate molecules to albumin, in solutions including this protein camptothecin rapidly loses its anticancer properties (Burke and Mi, 1994). Taking into consideration the above properties, the main criteria of new CPT analogues searching are: inhibition of topoisomerase I, low affinity of carboxylate form to albumin and high affinity of lactone form to cell membranes.

CPT and most of its analogues are fluorescent compounds so the methods of fluorescence spectroscopy are very useful in determining the biosystem properties. The methods let determine if the fluorescence molecules are bound or not to a big molecules (e.g. albumin) or other structures (e.g. liposomes or cells). Fluorescence emitted by small, fast-rotating molecules (e.g. free molecules of camptothecin) is strongly depolarised—the anisotropy coefficient has value close to 0. The anisotropy of fluorescence light emitted by big, slow-rotating molecules (or molecules bound to a big molecules of albumin or membranes) is close to fundamental anisotropy—the anisotropy coefficient is big. The measurements of fluorescence anisotropy let to determine the magnitude of affinity of CPT and its analogues to albumin and cell membranes. The knowledge of this parameters allows to predict how the studied compounds will operate in physiological conditions.

Many analogues of camptothecins have been synthesized. Among them particularly interesting is 7-*tert*-butyldimethylsil-10-hydroxycamptothecin, called DB-67. It exhibits strong affinity of lactone form to cell membranes and low affinity of carboxylate form to albumin. Such properties ensure high stability of DB-67

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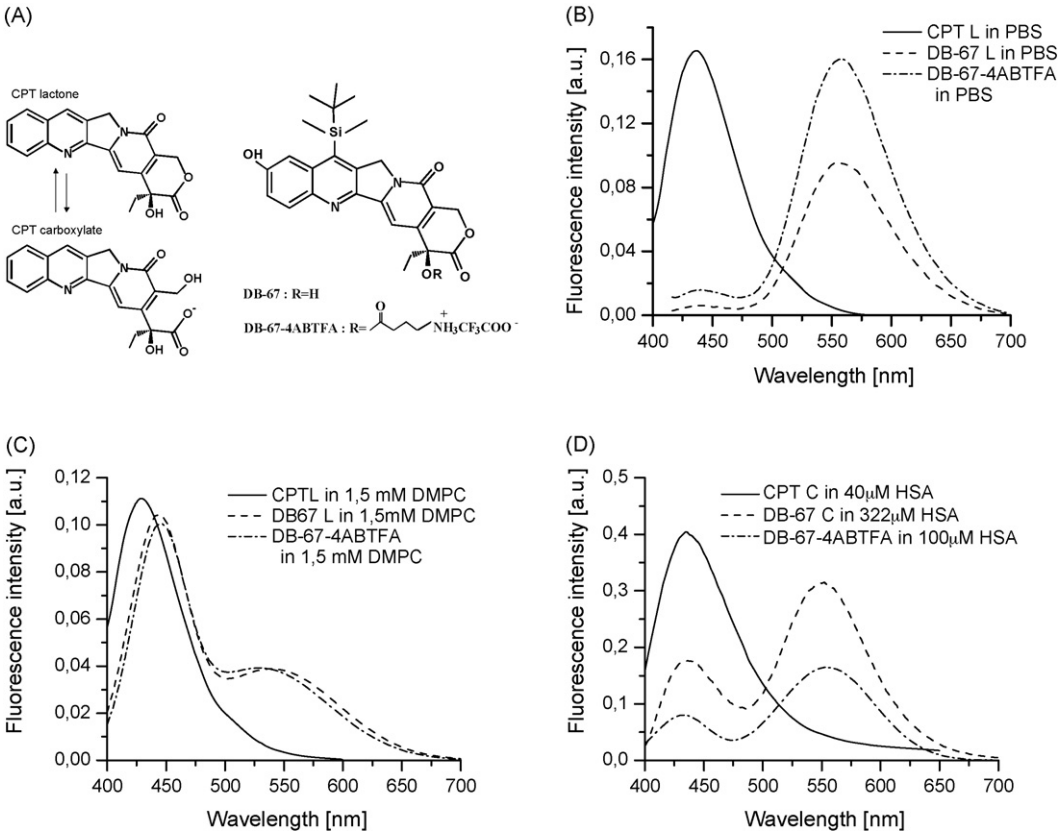

Fig. 1. Structures of the lactone and carboxylate forms of camptothecin, lactone form of DB-67 and ester DB-67-4ABTFA (A). Fluorescence emission spectra of CPT, DB-67 and DB-67-4ABTFA diluted in PBS (B), in liposomes suspension (C) and in HSA solution (D) at pH 7.4.

in blood and physiological fluids (Bom et al., 2000). The chemical structure of this compound is presented in Fig. 1A. It shows that DB-67 was obtained by substitution of hydrogen atoms in positions 7 and 10 of CPT molecule with the groups $–Si(CH_3)_2C(CH_3)_3$ and –OH, respectively. Because of their biophysical properties DB-67 seems to be one of the most promising analogues of CPT. It is a subject of intensive pharmacological researches *in vivo*. The clinical studies of DB-67 has been also started (Bence et al., 2004).

Despite of many advantages DB-67 has one major defect—it is weakly soluble in water, what seriously limits the possibilities of administration of this drug. One of methods of overcoming of this problem is formulation of DB-67 and administration in liposomal form. Another method is esterification of DB-67, i.e. obtaining water soluble ester of DB-67—prodrug, which in human body undergoes deesterification and transforms into active drug, DB-67. One of such esters, DB-67-4ABTFA (trifluoroacetic acid salt of 20(*S*)-aminobutyrate substituted DB-67) is the subject of this study. The analogue was obtained through replacing (in esterification process) of hydrophilic group in position 20(S) of DB-67 by lipophilic group: $–COO(CH_2)_3NH_3^+CF_3COO^-$ (Fig. 1A). The compound exists only in lactone form.

In the following chapters of this paper the fluorescence spectra of CPT, DB-67 and DB-67-4ABTFA diluted in PBS, HSA solution and in liposomes suspension are presented. The fluorescence anisotropy of these compounds in human plasma and liposomes was also measured. Using the results of these measurements the behavior of CPT, DB-67 and DB-67-4ABTFA in plasma and model membranes suspension is considered qualitatively and quantitatively. On the basis of changes of fluorescence spectra of prodrug diluted in PBS the transformation of DB-67-4ABTFA into DB-67 is discussed. The quantitative measure of drug affinity to protein or membranes is association constant. The values of association constants of studied compounds to albumin and liposomes calculated on the basis of fluorescence anisotropy measurements are presented in this work. The consequences resulted from the differences in properties of CPT, DB-67 and DB-67-4ABTFA are discussed.

2. Experimental

2.1. Materials

The samples of camptothecin and DB-67 (7-*tert*-butyldimethylsilyl-10-hydroxycamptothecin) were obtained from the laboratory of biotechnology, College of Pharmacy, University of Kentucky (Lexington, USA) and DB-67-4ABTFA (trifluoroacetic acid salt of 20(*S*)-aminobutyrate substituted DB-67) from Tigen Pharmaceuticals (Lexington, USA). To prepare 2 mM stock lactone solutions the camptothecins were dissolved in dimethylsulfoxide (DMSO, C_2H_6OS). 1 mM stock solutions of camptothecin and DB-67 carboxylate forms were obtained by dilution of stock lactone solutions in phosphate buffered saline (PBS) at pH 10 in volume ratio 1:1. Human serum albumin (95–97%) was purchased from Sigma–Aldrich (USA - Poland). Dimyristoylphosphatidylcholine (DMPC) obtained from Avanti Polar Lipids (Alabaster, AL, USA) was used to form liposomes. Stock DMPC suspension in PBS at pH 7.4 and temperature 37 °C was prepared by Vortex mixing. Next, suspensions were sonicated for about 2 h using a bath sonicator (Sonic-5, POLSONIC, Poland) until optical clarity was obtained. The desired concentrations of lipids and HSA were obtained by adding the stock lipid suspension and HSA solution to PBS. The pH and temperature of these solutions were kept at 7.4 and 37 °C, respectively.

The human plasma was prepared by centrifuging whole blood for 10 min at 2000 rpm. The human blood was received from the local blood-donation center (Bydgoszcz, Poland). On the basis of diagnostic norms it was assumed that the concentration of albumin in obtained human plasma equals 806 μM. The plasma was diluted by adding it to PBS at pH 7.4.

For fluorescence anisotropy measurements, the concentration of CPT, DB-67 and DB-67-4ABTFA in final samples was equal to 3 μM. Plasma solutions of 20 μM HSA were used for studying the time evolution of steady-state fluorescence anisotropy of CPT, DB-67 and DB-67-4ABTFA in the presence of albumin. For fluorescence spectra recordings the concentration of drugs and HSA was 3 and 40 μM, respectively.